Hormonal Responses to Insulin Infusion in Diabetes Mellitus

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Summary. Twenty diabetic patients and fourteen normal volunteers received infusion of 2.4 U neutral porcine insulin/h until either the blood glucose level was stable, or until hypoglycaemia occurred. As previously reported [1] in the normal group the blood glucose stabilised at 2.8 ± 0.1 mmol/l without any hypoglycaemic symptoms. There was an increase in blood levels of glucagon, cortisol and growth hormone as the blood glucose level fell, the mean peak increments being 167 ± 33 pg/ml, 400 ± 71 nmol/l and 29 ± 7 mU/l, respectively. In ten of the diabetic subjects (Group A) the blood glucose level stabilised at 3.6 ± 0.2 mmol/l during the insulin infusion, with peak increments in plasma glucagon (110 ± 24 pg/ml), cortisol (411 ± 71 nmol/l) and growth hormone (22 ± 6 mU/l), not significantly different from those in the normal subjects. These rises in hormone levels occurred during the last hour of infusion after normoglycaemia was reached and maintained. The ten remaining diabetics (Group B) developed symptoms of hypoglycaemia during the infusion. The peak increments in plasma glucagon (19 ± 7 pg/ml), cortisol (183 ± 36 nmol/l) and growth hormone (6 ± 2 mU/l) in this latter group were significantly less than those in the other diabetic group or the normals. The absence of counter-regulatory hormonal responses in the Group B diabetics was related to the development of hypoglycaemia and may be the result of a dysfunction of hypothalamic gluco-regulatory centres.

Key words: Low dose insulin infusion, blood glucose, glucagon, cortisol, growth hormone, autonomic neuropathy.

We have reported that some insulin-requiring diabetics, unlike normal subjects, become hypoglycaemic during prolonged low-dose (2.4 U/h) insulin

infusion [1]. This defect in counter-regulation is accompanied by impairment in glucagon release, even though the A cell remains capable of releasing glucagon during an arginine infusion. The similarity of this response to that in the denervated pancreas [2] led us to hypothesise that the defect was due to a selective form of diabetic autonomic neuropathy [3]. In the earlier communication we reported one untreated diabetic in whom the defect appeared following two years of insulin treatment.

We have enlarged our study from seven diabetic subjects to twenty and now include maturity onset diabetics on sulphonylurea therapy or on diet alone. Low dose insulin infusion (2.4 U neutral monocomponent porcine insulin/h) was used to elevate plasma insulin concentrations into the physiological range and the accompanying changes in plasma glucagon, cortisol and growth hormone were measured.

To ascertain whether the A cell was capable of glucagon release following a direct stimulus, the response to an arginine infusion was examined in those subjects in whom there was a defective glucagon response to hypoglycaemia.

Materials and Methods

Studies

All subjects were studied after an overnight fast. None received oral medication or subcutaneous insulin for twentyfour hours prior to the study. Intravenous cannulae were inserted in both antecubital fossae. After a rest period, 2.4 U neutral porcine insulin/h was infused in polygeline solution [4] via one cannula. Venous samples for plasma cortisol, growth hormone, glucagon, C-peptide immunoreactivity and blood glucose were withdrawn every 20 min via the other cannula in eleven subjects. In the remaining subjects, the same samples were withdrawn continuously via a double lumen catheter. The infusion ceased when there was a sustained plateau in blood glucose for twenty minutes or when symptomatic hypoglycaemia occurred (subjective symptoms with sweating and/or tachycardia).

Table 1. Clinical details of the diabetic subjects. "Good" clinical control was judged on satisfactory random outpatient blood glucose measurements, without severe hypoglycaemia during the preceding twelve months. (Mean random blood glucose = 5.9 ± 0.4 mmol/l); "Brittle" clinical control was based on alternate high blood glucose levels and severe hypoglycaemia during the preceding twelve months, despite careful treatment. (Mean random blood glucose = 11.0 ± 2.2 mmol/l)

	Group A Group B	
	n = 10	n = 10
Insulin requiring diabetics	2	7
Maturity onset diabetics –		
(a) Diet and/or tablets	6	1
(b) Recent tablet failure	2	2
Age (years)	41 ± 4	49 ± 5 NS
Weight (% ideal body weight)	105 ± 2	103 ± 2 NS
Sex – Males	6	6
Females	4	4
Duration of diabetes (years)	8 ± 2	8 ± 2
Type of clinical complications		
(a) Retinopathy	1	3
(b) Peripheral neuropathy	1	3
(c) Peripheral vascular disease	0	2
No known clinical complications	8	6
Clinical control – "good"	10	7
"brittle"	0	3

On a separate occasion, fifteen of the subjects received an infusion of arginine monochloride (0.25 g/kg in 20 min) and samples were collected every ten minutes for plasma glucagon and blood glucose estimation. Blood glucose was measured on a glucose analyser (Yellow Springs 23AM). Plasma cortisol was measured by competitive protein binding assay [5]; growth hormone [6] and glucagon were measured by radioimmunoassay, the latter using antiserum 30K supplied by Dr. R. Unger, a porcine standard (Novo Research Institute) and a charcoal separation technique. Plasma C-peptide reactivity was determined by radioimmunoassay [7] using reagents supplied by Novo Research Institute, Denmark.

Statistical comparisons were made using Wilcoxon's test (Documenta Geigy Scientific Tables, 1970). Results are presented as mean $\pm$ SEM.

Subjects

Fourteen normal and twenty diabetic volunteers, all within 10% of ideal body weight (Metropolitan Life Insurance Tables, 1959) gave informed consent to the studies. The mean age of the two women and twelve men in the normal group was 22 ± 2 (range 19–26) years. None had a family history of diabetes. There were eight women and twelve men in the diabetic group with a mean age of 44 ± 5 (range 18–67 years). The mean duration of diabetes was 8 ± 2 years (range 2 weeks to 17 years). Three diabetics were on diet alone; four on long term treatment with sulphonylureas (mean duration 10 ± 3 years); seven on insulin therapy (mean duration 8 ± 2 years); four recently commenced insulin after failure of oral

Fig. 1. Mean $\pm$ SEM blood glucose and plasma immunoreactive glucagon (IRG) levels in normal and diabetic subjects during the final two hours of infusion of 2.4 U neutral insulin/h. Diabetic subjects are divided into Groups A and B according to the pattern of blood glucose response

antidiabetic therapy (mean duration sulphonylurea treatment 8 ± 4 years); two were newly-diagnosed insulin requiring diabetics. No subject was receiving other medications at the time of the study. Two patients had peripheral vascular disease. Fourteen had no clinically detected complications. None had either symptoms of autonomic neuropathy (upper or lower gastrointestinal tract symptoms, impotence, "hypoglycaemic unawareness", sweating disorders, urinary tract symptoms) or postural hypotension. Three were considered clinically "brittle"; the remainder were considered to be in good control. No subject had known liver or renal impairment. The diabetics were divided into two groups, (see Table 1) on the basis of their blood glucose response to insulin infusion.

The two groups did not differ significantly in age, weight, sex distribution or duration of diabetes. There were more insulin requiring and less maturity-onset diabetics in Group B than A. There were more patients with complications in Group B, although six of the ten in this group had no known complications. The three so-called "brittle" diabetics were in Group B.

Results

The initial blood glucose was 4.0 ± 0.1 mmol/l in the normal subjects and 10.3 ± 1.0 mmol/l and 10.0 ± 1.0 mmol/l in Groups A and B respectively. The infusion time in normal subjects was 120 minutes. The mean time of infusion in the diabetic groups A and B did not differ significantly (235 ± 23 min [range 192–300 min] and 182 ± 24 min [range 80–309 min] respectively, $p > 0.05$).

Table 2. Blood glucose levels during infusion of 2.4 μ insulin per hour in the normal and diabetic groups. The rates of fall in blood glucose were measured over approximately twenty to sixty minutes after the insulin infusion commenced in normal subjects. This varied slightly to avoid measurement when the blood glucose was levelling. In the diabetic group, the rate of fall was measured over an equivalent blood glucose range (i.e. approximately 4 to 3 mmol/l)

	Normals	Diabetics	
		Group A	Group B
	n = 14	n = 10	n = 10
Initial blood glucose (mmol/l) (at commencement of insulin infusion)	4.0 $\pm$ 0.1	10.3 $\pm$ 1.0 ^c	10.0 $\pm$ 0.1 ^c
Rate of fall in blood glucose (mmol/l/h)	2.1 $\pm$ 0.2	2.7 $\pm$ 0.5 NS	2.9 $\pm$ 0.4 ^a
Final blood glucose (mmol/l)	2.8 $\pm$ 0.1	3.6 $\pm$ 0.2 ^c	2.1 $\pm$ 0.2 ^b

^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$, diabetics vs normals
NS = No significant difference

The mean blood glucose and plasma glucagon levels in the normals during the entirety of the insulin infusion and in the diabetics during the last two hours of infusion are shown in Figure 1.

As previously reported, while receiving 2.4 U neutral insulin/h no normal subject became clinically hypoglycaemic. There was a plateau in blood glucose at a mean level of 2.8 $\pm$ 0.2 mmol/l in the normals.

In ten of the diabetics (Group A) the response was similar to that of the normal subjects, although the plateau occurred at a significantly higher level than in the normals (3.6 $\pm$ 0.2 mmol/l, $p < 0.001$) and none became hypoglycaemic. However, in the ten Group B diabetics the blood glucose level fell steadily until clinical hypoglycaemia occurred and the infusion was terminated. The mean final blood glucose concentration in the latter group was 2.1 $\pm$ 0.2 mmol (significantly lower than in the normals, $p < 0.01$). The changes in blood glucose in the three groups are summarised in Table 2.

The mean initial plasma glucagon, cortisol and growth hormone levels in the normal subjects (74 $\pm$ 14 pg/ml, 294 $\pm$ 26 nmol/l and 8 $\pm$ 3 mU/l respectively) did not differ significantly from those in the whole diabetic group (51 $\pm$ 11 pg/ml, 366 $\pm$ 46 nmol/l, 8 $\pm$ 2 mU/l respectively, $p > 0.05$). The mean cortisol and growth hormone levels during the last two hours of infusion in the normal and diabetic

Fig. 2. Mean $\pm$ SEM plasma cortisol levels in thirteen normal and twenty diabetic subjects during the final two hours of infusion of 2.4 U neutral insulin/h; diabetic subjects are divided into Groups A and B according to the pattern of blood glucose response (see Figure 1)

groups are shown in Figure 2 and Figure 3 respectively.

The mean peak increments (i.e. the difference between the nadir and peak hormone level during the last sixty minutes of infusion) in glucagon, cortisol and growth hormone are detailed in Table 3.

The peak hormone increments of the normals and Group A did not differ significantly ($p > 0.05$). However, the peak increments in these hormones in Group B were significantly less than in the normals ($p < 0.001$, $p < 0.025$, $p < 0.01$ respectively) or in Group A ($p < 0.01$). There was no correlation between the rate of fall in glucose and the rises in individual hormones in normals or diabetics. There was a significant correlation between the increments in growth hormone and cortisol in the normal group ($r = 0.65$, $p < 0.01$) and between growth hormone and glucagon increments in diabetics ($r = 0.88$, $p < 0.001$). The correlations between the other hormone increments were not significant ($p > 0.05$).

Arginine infusion, performed in six of the diabetics in Group B, resulted in a mean peak rise in glucagon of 145 $\pm$ 23 pg/ml. This did not differ significantly from that in nine normal volunteers during arginine infusion (137 $\pm$ 32 pg/ml, $p > 0.05$).

In the eight diabetics in Group A in whom the plasma C-peptide reactivity was measured, the mean initial level was 1.50 $\pm$ 0.28 ng/ml. In four of the

Fig. 3. Mean $\pm$ SEM plasma growth hormone levels in fourteen normal and twenty diabetic subjects during the final two hours of infusion of 2.4 U neutral insulin/h; diabetic subjects are divided into Groups A and B according to the pattern of blood glucose response (see Figure 1)

Table 3. The mean $\pm$ SEM peak increments in plasma glucagon, cortisol and growth hormone in normal and diabetic subjects during infusion of 2.4 U neutral insulin/h. The peak increment was the difference between the nadir and the peak hormone level during the last hour of infusion. Each diabetic group is compared with the normals (^a) and Group B is compared with Group A (^d)

	Normals	Diabetic subjects	
		A (n = 10)	B (n = 10)
Peak glucagon increment (pg/ml)	(n = 8) 167 $\pm$ 33	110 $\pm$ 24 NS	19 $\pm$ 7 ^{c d}
Peak cortisol increment (nmol/l)	(n = 13) 400 $\pm$ 71	411 $\pm$ 71 NS	183 $\pm$ 36 ^{a d}
Peak growth hormone increment (mU/l)	(n = 14) 29 $\pm$ 7	22 $\pm$ 6 NS	6 $\pm$ 2 ^{b d}

^a $p < 0.025$; ^b $p < 0.01$; ^c $p < 0.001$; ^d $p < 0.01$

eight diabetics in Group B in whom serum C-peptide reactivity was measured the level was below assay sensitivity; in the remainder the mean initial level was 1.02 ± 0.32 ng/ml. The mean final levels during insulin infusion in Group A and in those in Group B who had measurable C-peptide reactivity were not significantly different (0.34 ± 0.11 ng/ml and 0.66 ± 0.14 ng/ml respectively, $p > 0.05$).

Discussion

In Group A diabetics receiving an infusion of 2.4 U neutral insulin/h there were rises in serum levels of glucagon, cortisol and growth hormone during the last hour of infusion. In these subjects, as in the normal group, there was a plateau in blood glucose level despite continuing insulin infusion.

In Group B diabetics, who were matched for age, sex distribution, weight and duration of diabetes with Group A, the blood glucose level fell until clinical hypoglycaemia occurred at 2.1 ± 0.2 mmol/l. The inability of Group B diabetics to prevent hypoglycaemia during insulin infusion was accompanied by increments in serum glucagon, cortisol and growth hormone which were significantly less than those in the normal subjects or in the other diabetic group, despite a lower final blood glucose. Neither the type of diabetes nor its treatment was directly related to this counter-regulatory impairment. As the three "brittle" diabetics in this series were in Group B, it is possible that the counter-regulatory defect may be a factor adversely affecting diabetic control.

However, it is difficult to attribute glucose counter-regulation to the release of a single hormone and, during insulin hypoglycaemia, the hepatic glucose output has been shown to increase before any hormone elevation occurs [8]. Thus the hormonal changes reported here are part of the counter-regulatory response, not the whole response. It is probable that there is an impairment in catecholamine release in some diabetics similar to that of glucagon, cortisol and growth hormone [9]. Although the diabetic group was older than the normals, there is no evidence that counter-regulatory ability declines with age. There was no direct relationship between the subjects' glucagon responsiveness and their basal C-peptide levels.

The presence of a glucagon response to arginine in the patients tested in Group B is evidence of the presence of functional A cells, despite the low glucagon response during insulin infusion. Glucagon release during minor hypoglycaemia is under vagal control from a central glucoreceptor [10, 11] rather than under direct control by circulating glucose levels. Our previous hypothesis that the impaired glucagon response was due to a selective form of autonomic neuropathy [3] cannot, however, explain the impaired responses in cortisol and growth hormone. Two possible explanations are advanced. A lesion in the hypothalamic centre(s) responsible for gluco-regulation could result in the hypoglycaemic unresponsiveness described. The unimpaired awareness of hypoglycaemia in all ten affected diabetics would

suggest that the central gluco-receptors themselves are intact. Other autonomic disturbances which have been described in diabetes – such as nocturnal sweating [12], autonomic overactivity [13] and pyrexia of hypoglycaemia [14] could be the result of central autonomic nervous system dysfunction. Alternately, a defect in the function of a neurotransmitter common to the central and peripheral nervous system could explain our findings. For example, serotonin has been implicated in the release of growth hormone [15], adrenocorticotrophic hormone [16] and glucagon [17]. Its concentration in the hypothalamus has been shown to be increased during insulin-induced hypoglycaemia in the rat [18].

In Group A, the final blood glucose level (3.6 ± 0.2 mmol/l) was higher than that in the normals (2.8 ± 0.1 mmol/l, $p < 0.001$). It is possible that the diabetics may be less sensitive to insulin action than the normal subjects [19] and the greater age of the diabetics may contribute to this. However, decreased sensitivity appears unlikely, as the rate of fall of blood glucose in the diabetics was not lower than that in the normal subjects. Alternately, it may be that the higher final blood glucose level is due to an inappropriately early release of counter-regulatory hormones in this diabetic group.

Others have reported early rises in cortisol and growth hormone in ketoacidotic patients within three hours of commencement of insulin infusion and while the patients were still moderately hyperglycaemic [20, 21]. However, the hormonal changes reported in this paper occurred in stable diabetics after normoglycaemia was attained and after approximately three hours of insulin infusion.

Further studies are required to test the hypothesis. However, our findings are consistent with reports from other workers of the unexplained absence or impairment of growth hormone [22] or cortisol [23] responses to hypoglycaemia in some diabetics. We have shown that some diabetics lack a counter-regulatory response capable of preventing hypoglycaemia during low-level insulin infusion and that this is accompanied by lesser increments in glucagon, cortisol and growth hormone than are seen in normals. We propose that this is due to an acquired defect in counter-regulatory control, probably of central origin.

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References

1. Campbell, L. V., Kraegen, E. W., Lazarus, L., Meler, H., Chia, Y. O., Chisholm, D., Alford, F.: Defective hypoglycaemia counter-regulation during physiological insulin infusion. In: Abstracts IX Congress of the International Diabetes Federation. Bajaj, J. S., (Ed.), p. 35. Amsterdam, London: Excerpta Medica 1976
2. Beaven, D. W., Arcus, A. C., Hart, D. W., Holland, G. W.: Dissociated α and β cell responses in permanent pancreatic autotransplants. In: Abstracts IX Congress of the International Diabetes Federation. Bajaj, J. S. (Ed.), p. 34. Amsterdam, London: Excerpta Medica 1976
3. Campbell, L. V., Kraegen, E. W., Lazarus, L.: Hypothesis: Defective blood glucose counter-regulation in diabetics is a selective form of autonomic neuropathy. *Br. Med. J.* **1977** *II*, 1527–1529
4. Kraegen, E. W., Lazarus, L., Meler, H., Campbell, L. V., Chia, Y. O.: Carrier solutions for low-level insulin infusion. *Br. Med. J.* **1975** *III*, 464–466
5. Murphy, B. E. P.: Some studies of the protein binding of steroids and their application to the routine micro and ultra micro measurement of various steroids in body fluids by competitive protein-binding radioimmunoassay. *J. Clin. Endocrinol. Metab.* **27**, 973–990 (1967)
6. Molinatti, G. M., Mossara, F., Pennisi, F., Scassellati, G. A., Strumie, E., Vancheri, L.: Radioimmunoassay of growth hormone. *J. Nucl. Biol. Med.* **13**, 26–36 (1969)
7. Heding, L. G., Larsen, U. D., Markussen, J., Jorgensen, K. H., Hallund, O.: Radioimmunoassays for human, pork and ox C-peptides and related substances. *Horm. Metab. Res.* **5** (Suppl.), 40–44 (1973)
8. Sacca, L., Sherwin, R., Cryer, P., Felig, P.: Role of blood glucose concentration in the hormonal control of hepatic glucose production in vivo: evidence of autoregulation by glucose. *Diabetologia* **14**, 267 (1978)
9. Rosak, C., Vogel, D., Brecht, H. M., Althoff, P., Neubauer, M., Haupt, E., Schulz, F., Jungmann, E., Schöffling, K.: Evaluation of hormonal and metabolic events following insulin induced hypoglycaemia. *Diabetologia* **14**, 266 (1978)
10. Russell, R. C. G., Thomson, J. P. S., Bloom, S. R.: The effect of truncal and selective vagotomy on the release of pancreatic glucagon, insulin and enteroglucagon. *Br. J. Surg.* **61**, 821–824 (1974)
11. Bloom, S. R., Vaughan, N. J. A., Russell, R. C. G.: Vagal control of glucagon release in man. *Lancet* **1974** *II*, 546–549
12. Rundles, R. W.: Diabetic neuropathy. General review with report of 125 cases. *Medicine (Baltimore)* **24**, 111–160 (1945)
13. Martin, M. M.: Involvement of autonomic nerve-fibres in diabetic neuropathy. *Lancet* **1953** *I*, 560–565
14. Chochinov, R., Daughaday, W. H.: Marked hyperthermia as a manifestation of hypoglycaemia in long-standing diabetes mellitus. *Diabetes* **24**, 859–860 (1975)
15. Smythe, G. A., Lazarus, L.: Growth hormone regulation by melatonin and serotonin. *Nature* **244**, 230–231 (1973)
16. Plonk, J. W., Bivens, C. H., Feldman, J. H.: Inhibition of hypoglycaemia-induced cortisol secretion by the serotonin antagonist cyproheptadine. *J. Clin. Endocrinol. Metab.* **38**, 836–840 (1974)
17. Campbell, L. V., Smythe, G. A., Kraegen, E. W., Lazarus, L.: Biogenic amines and glucagon release (Abstr.). *Aust. N. Z. J. Med.* **7**, 686–687 (1977)
18. Gordon, A. E., Meldrum, B. S.: Effect of insulin on brain 5-hydroxytryptamine and 5-hydroxy-indole-acetic acid of rat. *Biochem. Pharmacol.* **19**, 3042–3044 (1970)

19. Reaven, G. M., Silvers, A., Farquhar, J. W.: Study of the relationship between plasma insulin concentration and efficiency of glucose uptake in normal and mildly diabetic subjects. *Diabetes* **19**, 571–578 (1970)
20. Alberti, K. G., Hockaday, T. D.: Diabetic Coma: serum growth hormone before and during treatment. *Diabetologia* **9**, 13–19 (1973)
21. Sönksen, P. H., Srivastava, M. C., Tompkins, C., Nabarro, J. D. N.: Growth hormone and cortisol responses to insulin infusion in patients with diabetes mellitus. *Lancet* **1972 II**, 155–160
22. Molnar, G. D., Fatourehchi, V., Ackerman, E., Taylor, W. F., Rosevear, J. W., Gatewood, L. C., Service, F. J., Moxness, K. E.: Growth hormone and glucose interrelationships in diabetes: studies of inadvertent hypoglycaemic episodes during continuous blood glucose analysis. *J. Clin. Endocrinol. Metab.* **32**, 426–437 (1971)
23. Serio, M., Tarquini, B., Contini, P., Bucalossi, A., Toccafondi, R.: Plasma cortisol response to insulin and circadian rhythm in diabetic subjects. *Diabetes* **17**, 124–126 (1968)

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